WO 2005/009350 PCT/US2004/021725

FIGURE 1 HSP 90-beta (Mouse) SEQ ID NO:1 1 mpeevhhgee evetfafqae iaqlmsliin tfysnkeifl relisnasda ldkiryeslt 61 dpskldsgke lkidiipnpq ertltlvdtg igmtkadlin nlgtiaksgt kafmealqag 121 adismigqfg vgfysaylva ekvvvitkhn ddeqyawess aggsftvrad hgepigrgtk 181 vilhlkedat eyleerrvke vvkkhsafig ypitlyleke rekeisddea eeekgekeee 241 dkddeekpki edvgsdeedd sgkdkkkktk kikekyidge elnktkpiwt rnpdditgee 301 ygefyksltn dwedhlavkh fsvegqlefr allfiprrap fdlfenkkkk nniklyvrrv 361 fimdscdeli peylnfirgv vdsedlplni sremlqqski lkvirknivk kclelfsela 421 edkenykkfy eafsknlklg ihedstnrrr lsellryhts qsgdemtsls eyvsrmketq 481 ksiyyitges keqvansafv ervrkrgfev vymtepidey cvqqlkefdg kslvsvtkeg 541 lelpedeeek kkmeeskakf enlcklmkei ldkkvekvti snrlvsspcc ivtstygwta 601 nmerimkaqa 1rdnstmgym makkhleinp dhpivetlrq kaeadkndka vkdlvvllfe 661 tallssgfsl edpqthsnri yrmiklglgi dedevaaeep naavpdeipp legdedasrm 721 eevd HSP 90-beta (Human) SEO ID NO:2 1 mpeevhhgee evetfafqae iaqlmsliin tfysnkeifl relisnasda ldkiryeslt 61 dpskldsgke lkidiipnpq ertltlvdtg igmtkadlin nlgtiaksgt kafmealqag 121 adismigqfg vgfysaylva ekvvvitkhn ddeqyawess aggsftvrad hgepigrgtk 181 vilhlkedat eyleerrvke vykkhsafig ypitlyleke rekeisddea eeekgekeee 241 dkddeekpki edvgsdeedd sgkdkkktk kikekyidge elnktkpiwt rnpdditgee 301 ygefyksltn dwedhlavkh fsvegqlefr allfiprrap fdlfenkkkk nniklyvrrv 361 fimdscdeli peylnfirgv vdsedlplni sremlqqski lkvirknivk kclelfsela 421 edkenykkfy eafsknlklg ihedstnrrr lsellryhts qsgdemtsls eyvsrmketq 481 ksiyyitges keqvansafv ervrkrgfev vymtepidey cvqqlkefdg kslvsvtkeg 541 lelpedeeek kkmeeskakf enlcklmkei ldkkvekvti snrlvsspcc ivtstygwta 601 nmerimkaga lrdnstmgym makkhleinp dhpivetlrq kaeadkndka vkdlvvllfe 661 tallssgfsl edpqthsnri yrmiklglgi dedevaaeep naavpdeipp legdedasrm 721 eevd HSP 90-alpha (Human) SEQ ID NO:3

1 mpeetqtqdq pmeeeevetf afqaeiaqlm sliintfysn keiflrelis nssdaldkir 61 yesltdpskl dsgkelhinl ipnkqdrtlt ivdtgigmtk adlinnlgti aksgtkafme 121 alqagadism igqfgvgfys aylvaekvtv itkhnddeqy awessaggsf tvrtdtgepm 181 grgtkvilhl kedqteylee rrikeivkh sqfigypitl fvekerdkev sddeaeeked 241 keeekekeek esedkpeied vgsdeeekk dgdkkkkki kekyidqeel nktkpiwtrn 301 pdditneeyg efyksltndw edhlavkhfs vegqlefral lfvprrapfd lfenrkkknn 361 iklyvrrvfi mdnceelipe ylnfirgvvd sedlplnisr emlqqskilk virknlvkkc 421 lelftelaed kenykkfyeq fskniklgih edsqnrkkls ellryytsas gdemvslkdy 481 ctrmkenqkh iyyitgetkd qvansafver lrkhgleviy miepideycv qqlkefegkt 1vsvtkegle lpedeeekkk qeekktkfen lckimkdile kkvekvvvsn rlvtspcciv 601 tstygwtanm erimkaqalr dnstmgymaa kkhleinpdh siietlrqka eadkndksvk 661 dlvillyeta llssgfsled pqthanriyr miklglgide ddptaddtsa avteempple

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HSP 84 (Mouse) SEQ ID NO:4

1 mpeevhhgee evetfafqae iaqlmsliin tfysnkeifl relisnasda ldkiryeslt dpskldsgke lkidiipnpq ertltlvdtg igmtkadlin nlgtiaksgt kafmealqag lgmtkadlin nlgtiaksgt kafmealqag ddeqyawess aggsftvrad hgepigrgtk ekvvitkhn ddeqyawess aggsftvrad hgepigrgtk vilhlkedqt eyleerryke evykkhsqfig ypitlyleke rekeisddea eeekgekeee sgkdkkktk kikekyidqe elnktkpiwt rnpdditqee fsvegqlefr allfiprrap fdlfenkkk nniklyvrrv dsedlplni sremlqqski lkvirknivk kclelfsela egekenykkfy eafsknlklg ihedstnrrr lsellryhts qsgdemtsls eyvsrmketq ervrkrgfev vymtepidey cvqqlkefdg kslvsytkeg ldkkvekvti snrlysspcc ivtstygwta dhpivetlrq kaeadkndka vkdlvvllfe dedevtaeep saavpdeipp legdedasrm

721 eevd

HSP 84 (Human) SEO ID NO:5

1 mpeevhhgee evetfafqae iaqlmsliin tfysnkeifl relisnasda ldkiryeslt dpskldsgke lkidiipnpq ertltlvdtg igmtkadlin nlgtiaksgt kafmealqag lgmtkadlin nlgtiaksgt kafmealqag lgmtkallin nlgtiaksgt kafmealqag lgmtkallin nlgtiaksgt lgmtkallin nlgtiaksgt kafmealqag lgmtkallin nlgtiaksgt lgmtkallin nlgtiaksgt lgmtkallin nlgtiaksgt lgmtkalli

HSP 86 (Mouse) SEQ ID NO:6

1 mpeetqtqdq pmeeevetf afqaeiaqlm sliintfysn keiflrelis nssdaldkir 1981dpskl dsgkelhinl ipskqdrtlt ivdtgigmtk adlinnlgti aksgtkafme 121 alqagadism igqfgvgfys aylvaekvtv itkhnddeqy awessaggsf tvrtdtgepm 181 grgtkvilhl kedqteylee rrikeivkkh sqfigypitl fvekerdkev sddeaeekee 241 keeekekeek esddkpeied vgsdeeeek kdgdkkkkk ikekyidqee lnktkpiwtr 301 npdditneey gefyksltnd weehlavkhf svegqlefra llfvprrapf dlfenrkkkn 191 remlqqskil kvirknlvkk eylnfirgvv dsedlplnis remlqqskil kvirknlvkk sellryytsa sgdemvslkd yctrmkenqk hiyfitgetk dqvansafve rlrkhglevi ymiepideyc vqqlkefegk 192 tlvsvtkegl elpedeeekk kqeekktkfe nlckimkdil ekkvekvvvs nrlvtspcci aeadkndksv 661 kdlvillyet allssgfsle dpqthanriy rmiklglgid eddptvddts aavteemppl

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HSP 86, HSP 60 (Human) SEQ ID NO:7

1 mlrlptvfrq mrpvsrvlap hltrayakdv kfgadaralm lqgvdllada vavtmgpkgr 61 tviieqswgs pkvtkdgvtv aksidlkdky knigaklvqd vanntneeag dgtttatvla 121 rsiakegfek iskganpvei rrgvmlavda viaelkkqsk pvttpeeiaq vatisangdk 181 eigniisdam kkvgrkgvit vkdgktlnde leiiegmkfd rgyispyfin tskgqkcefq 241 dayvllsekk issiqsiypa leianahrkp lviiaedvdg ealstlvlnr lkvglqvvav 301 kapgfgdnrk nqlkdmaiat ggavfgeegl tlnledvqph dlgkvgeviv tkddamllkg 361 kgdkaqiekr iqeiieqldv ttseyekekl nerlaklsdg vavlkvggts dvevnekkdr 421 vtdalnatra aveegivlgg gcallrcipa ldsltpaned qkigieiikr tlkipamtia 481 knagvegsli vekimqssse vgydamagdf vnmvekgiid ptkvvrtall daagvasllt 541 taevvvteip keekdpgmga mggmgggmgg gmf

L-plastin (Human) SEQ ID NO:8

1 margsvsdee mmelreafak vdtdgngyis fnelndlfka aclplpgyrv reitenlmat 181 eekyafvnwi nkalendpdc rhvipmnpnt nkkkltpfti qenlnlalns asaigchvvn igaedlkegk lrwanyhlen asaigchvvn dmsglrekdd igraecmlqq aerlgcrqfv tatdvvrgnp ekikvpvdwn rvnkppypkl ggnmkklenc gggqkvnddi vnwvnetlr eaeksssiss rkigarvyal fkdpkistsl pvldlidaiq pgsinydllk follog pedlvevnpk mvmtvfaclm

FIG. 2

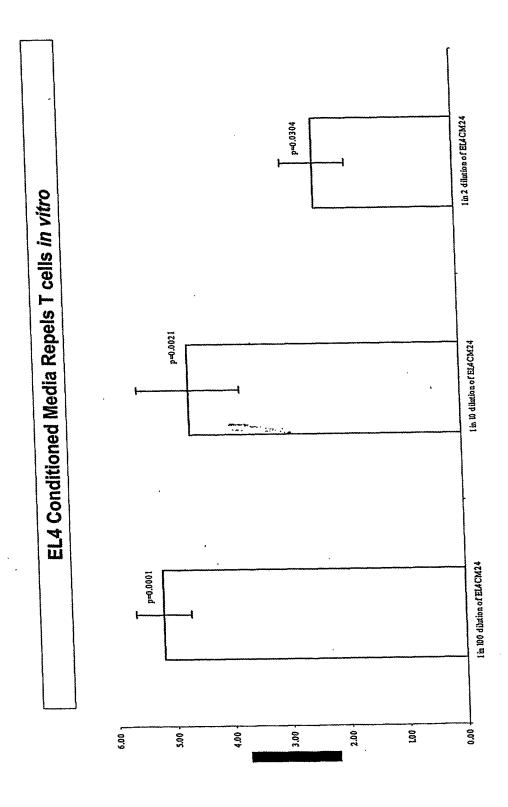
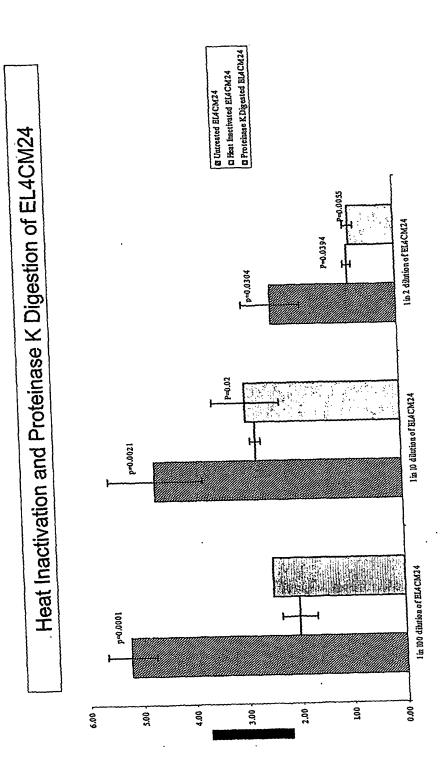


FIG. 3



Best Available Copy

1 in 2 dilution of ELACM 24

☑ Geldanamycin Treated EL4CM24

P=0.0304

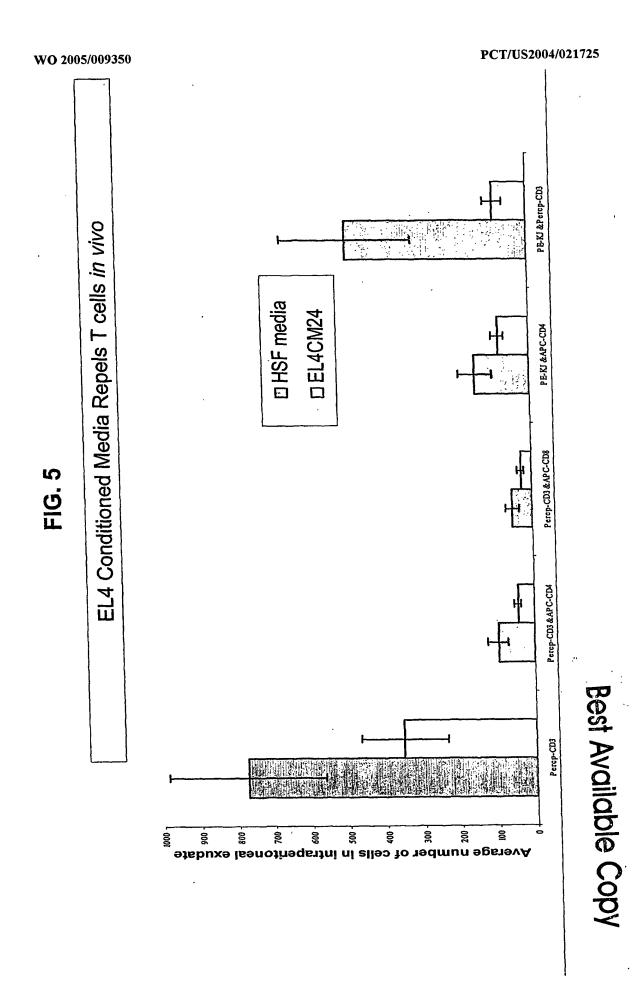
□ Pertussis toxin Treated cells ☐ Radicicol Treated EL4CM24

☑ Untreated EL4CM24

P=0.0021

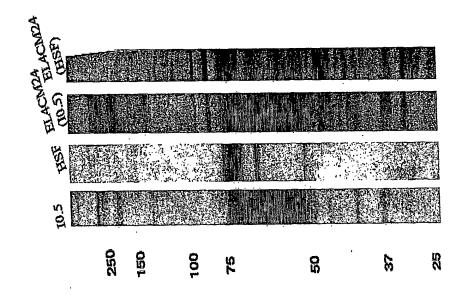
Use of Specific Inhibitors

**Best Available Copy** 



7/25

**FIG. 6** EL4CM24 SDS PAGE



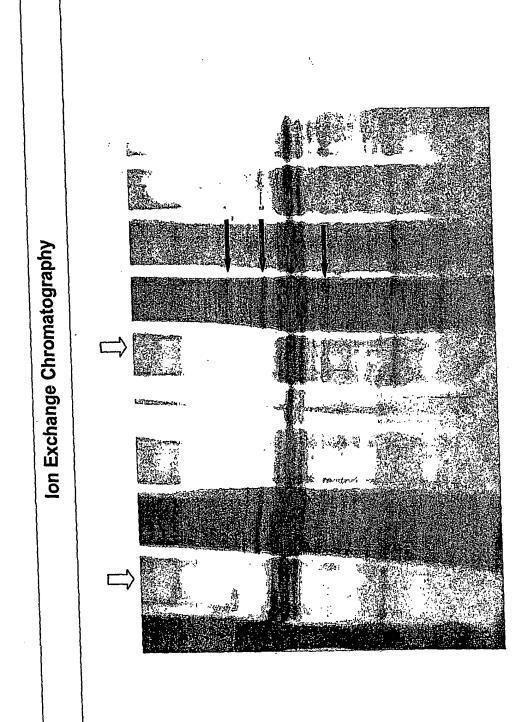
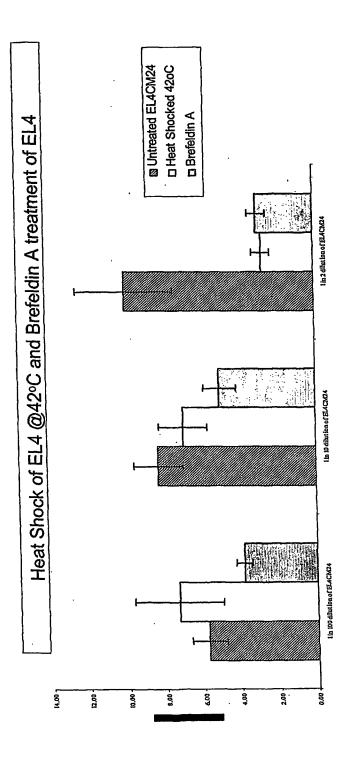
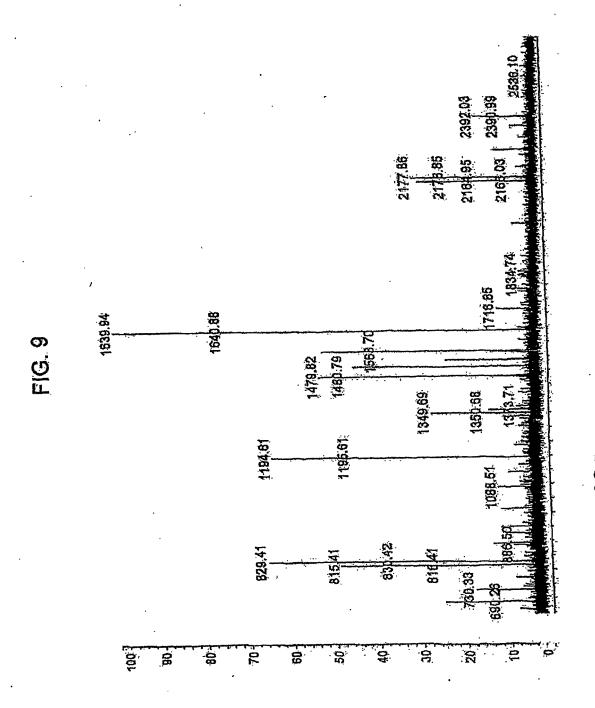


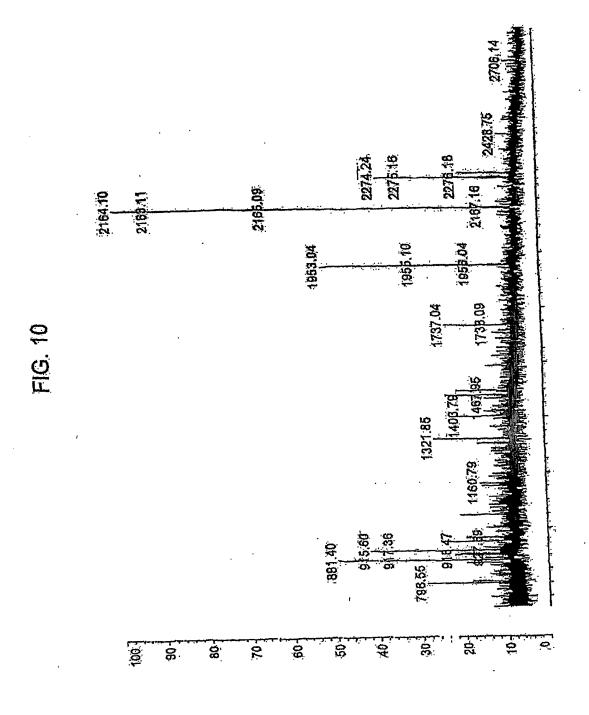
FIG. 7

FIG. 8

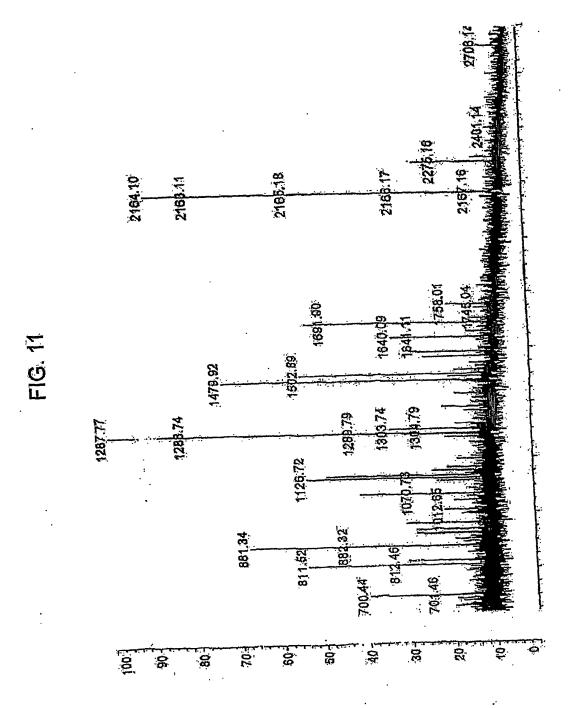


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12/25



# **FIG. 12A**

# **MS-Fit Search Results**

Press stop on your browser if you wish to abortahis MS-Fit search prematurely.

Sample 1D (comment): Magic Bullet digest
Database searched: NGBInt. 121002
Molecular weight search (1000 - 100000 Da) selects 1195692 entries.
Foil pf. range: 1247039 entries.
Species search (MARIMALS) selects 197947 entries.
Continued molecular weight, pl and species searches select 186900 entries.
MS-Fit search selects 407 ontries (results displayed for 10p 4 matches):

Considered modifications: [Peptido Naterminal Gla to pyroGla ) Oxidation of M | Protein Naterminus Acetylated |

Chiterocten.			•	, .,				
Min.# Peptides to Match	Peptide Mass Tolerance (+/-) 150,000	Pepride Masses are monoisotopic	Digest Uscă Trypsin	Max. # Missed Cleavages	Cysteines Modified by serylamide	Pepiido Niterminus Hydrogen (H)	Peptide Carminus Free Acid (O H)	Input # Poptido Masses IS

#### Result Summary

Rank	MOWSE Score	hantoped / Mrises H (50)	Protein MW (Day)pi	Species	Accession #	
2	6.79e+003 6.79e+003 2.25e+003	8/15 (53%) 8/15 (53%) 8/15 (53%) 1/15 (46%)	83264.6 / 4.97		20149594 11277141	heat shock protein 90 beta Unknown (protein for MGC:1138) hypothetical protein heat shock protein 90; hsp90

#### Detailed Results

1. 8/15 mutches (51%). 81963.2 Da. pl = 4.99. Acc. # 20177936. EQUUS CABALLUS, heat shock protein 90 bets .

1 . Of ( > titrem	+w.s y== -,~z-,-				· _	
	MR <sup>+</sup> matched	hba			Peptide Sequence	Modifications
689.3000	689,3946	-137.2410	570	575	(KO <u>VTISNR</u> (L)	
829.4100	879,5300	-144.6415	323	329	(R)ALLFIPR(R)	
891.3500	891,4152	-84.4094	421	427	(KOFYEAFSE(N)	
1194.6100	1194.6483	-32.0277	65	74	(K)IDHPNEOER(T)	
1348.6900	. 1348,6650	18.5557	312	323	(KORFSVEGOLEFICA)	
1513.7800	1513.7867	4.1036	.371	38	(R)GVVDSEDLPLNISR(R)	ends.
2176,8601	2176.945	7 -39.368	1 449	46	7 (R)YHTSOSCDEMTSLSEYYSR	(A-2)
2390.9900	<b>) 2391:183</b> :	2, -80.409	6 47	4 49	4 (K)SIYYITGESKEQVANSAFVE	77/. v

<sup>7</sup> minutohed masses: 730,3300 815,4100 1479,8200 1537,7700 1567,7000 1639,9400 1715,8900

The matched peptides cover. 13% (95/713 (Ass) of the protein. Coverage Map for This Hit (MS-Digest index ii): 1205/701

### **FIG. 12B**

```
2. 8115 marches (5354), 83164 6 Oo; pt = 4.97. / Acc. 20149594 HOMO SARRENS, Unknown Grotelin for MCC: 1138).
  mix mit, Delta start and Peptine Sequence
                                                                                                     Modisticacions
   689.3000 689.3946 437.2410 578 583 (K)VEISNECL)
   $29.4100 $29.5300 -144.6415 $31 337 (B)ALL-FIFTE(R)
   BULISON BULGES ALLONG CO 415 (K)PYHAPEKIN
  2390.9900 2391.1632 -80.8096 492 803 (KOSIYYITGI-SEEROYAISAFEER(V).
  Tenunatched masses: 730:3300 815 4100 1479.8200 1537.7700 1567.7000 1639.9400 1715.8900
   The matched peptidist cover 13% (93/724 AAS) of the protein. Coverage Map for Tide His (MS-Diggel index B): 137455.
   3. 4/15 matches (336). $1843.9 De. of = 5.26. Aco. # [1277141, HOMO SAPIENS, hypothetical provide
      m/z mis Delca start end Peplide Sequence submitted matched ppm
                                                                                                       Plodifications
      689,3000 689,3946 -137,2410 578 383 (GYTISNECL)
      829,4100 829,5300 -144,6419 331 337 (RJALLFIPRIR)
      891.3500 691.4252 -84.4094 429 435 (K)FYEAFSKIN)
     1394.6100 1346.643 - 32.0271 73 82 (K)MHPNPDER(T)
1348.6300 1346.645 - 32.0271 73 82 (K)MHPNPDER(T)
      1513,7800 1513,7502 -4.1036 379 393 (B)GYYDSEDIATECTER(B)
      2176.8600 2176.5457 -39.3681 457 475 (P)YHTEOSCOPMISLSEYYSR(M)
      TING SHOOT LIST -50.0006 402 TOL (KUNIYYTTUINKEOVARCAHYEKIY)
     7 unmatched master: 730,3100 813,4100 1479,5200 1527,7700 1567,7000 1639,9400 1715,8900
      The matched peptides cover 12% (957) 7 AA's) of the protein. Coverage Map for This Hit (MS-Digest lader 4): 1101216.
      4. 7/15 matches (46%) $72164 Da. pl - 5.06. Acc. # 1346320. RATTUS SP., base shock probible 90; https://
                                                                                                           Modifications
        mir. Bill' Delta start and Pepildo Sequence submitted matched ppm
         649.3040 689.3446 -137.2410 578 583 (KOVYYSHR(C)
         829.4100 829.5300 -144.6415 331 337 (R)ALLEIPE(R)
        ### 1.500 ### 2.4169 429 425 | KIEFFEASEKO

| 14.650 | 14.655 | 14.655 | 10 100 | KIEFFEASEKO

| 151.750 | 151.750 | 4.1016 | 379 | 191 | (RICVVOSROLFINISECO)

| 151.750 | 151.750 | 4.1016 | 379 | 191 | (RICVVOSROLFINISECO)

| 171.750 | 2176.9457 | 429.2657 | 427 | 427 | (RITTISUSCOMMINASECYSSEM)
         2390.9900 2391.1832 -80.8096 483 582 (K)SIVVITGESKEQVANSARVERICE)
        8 unmarched dissess: 730.3300 815.4100 1194.6100 1479.8200 1537.7700 1567.7000 1679.9460 1715.8900
                                                                         MS-Tag Search Results
          Sample ID (continent): Apr Act 1040 AUFVLEDLA
Children eranketh MCBlancili 1992.
Phil Madecular Wilden nagu: 1341037 endica.
Phil finneng: 11471034 confer.
Bedden esucch (MAKRILLS) publicai 197444 endita.
Camber of teagenon publing through parent unan filher: 4353
MS-Teg seinch seinem'? engine frenulus displayed for inp 3 munibus).
           Parent metri- 1194.6(00 (ef. 0.2090 De)
Fraginces (ons weed in earth: 175.31, 212.00, 229.20, 342.61, 355.43, 512.43, 519.50, 616.31, 713.44, 740.51, 166.33 (ef. 0.50 De)
         Am Types Considered: 9 8 8 9 8 8
            Search Mar. F. Peptide Messes Digest. Mes. S. Misted Cysteines Peptide Production are Uted Clearages Modified by Neumbus Cterminas Renthy Tomonisotopic Trypita errylandide Hydrogen (E): Free Acid (O II)
                                                                                  Result Summary
                                                                                                              NCBIact 22002: Protein Neme
                                                                                                Species
                                                      Celculated Error
(Da) (Da)
                                                                            MILY (Daypt
                                                                                                                               Heat thack protein HSF 90-bits (HSF $4)
(Trimer specific transplantation $4 kDs 2018pm)
(TSTA)
                                CONTINUED TIPLINE COST TIPLINE MOUSE
                                                                                                                  121681
                      D/II
              1
                                                                                                                  6680305
                                                                                                                                hest shock protein, $4 kD4 1
                                PRODUCED COST 1.186.6183 -0.0381 $1361.115.03 GUS MUSCULUS
                       0/13
                                                                                                                                heat shock protein bets
                                (K)(O)(PNPOER(T) 1194.6(3) -BOJES 14066.4/4.64 HOMO SAPIENS
                                                                                                                  2351110
```

**dest** 

# FIG. 12C

# **Detailed Results**

Rank	d Unmstehed	Sequence.	MH. Calculated		Protein MW (De)/pl	Spicits	NCBing 111001 Accession #	MS-Digest Tadex p	
١	6/11 (K)  ere	naoqra.ng	(L) 1134'6483 (Dv)	-0:0383 (De)	83325,774.93	MOUSE	<u> 12368).</u>	312126	Hear shock protein HSP 90-bria (HSP 84) (Tumor specific transplancation 84 kDa antigen) (TSTA)
	0/11 (K)	IDILPNPORR	(T) 1194,6483	-0:0381	83361.175.03	MUS MUSCULUS	6680305	5R3990	heat shock protoin, 44 kDs I
	Fragment-lon ( fou-type Dilto Da		0.10 0.08 DI 0.03	0.42 DIL 0.42	PQE   Y4-NH.		723.44 740.58 966 76-NHJ 76 74 040 0.21 0.1		hen stock protein beta .
•		y, 1	NP   b <sub>2</sub> -0.10   0.08 DI	<b>b</b> , [	PQE   SI-NH	y IDS-NH	723,44	i)	

#### MS-Tag Search Results

Sample (D. (comment): Ayo A-1 1040 AKPVLEDLR

Database searched: NCBlant 121001

Milecular weight search (1000 - 100000 Da) selects 1195692 entries.

Full pit range: 1747039 catries.

Spocies search (MAMMALS) solocts 197947 entries.

Combined molecular weight, pi and species searches select 196900 entries.

Number of sequences passing through parent mass filter: 4989

MS-Tag rearch selects 18 catries (results displayed for top 3 matches).

Parent mass: 815.4100 (+- 0.7000 DB)

Fragment fone used in search: 185.76, 255.77, 272.34, 298.32, 354.45, 371.53, 417.39, 445.25, 518.35 (+- 0.50 Da)

fon Types Considered: a b B y n h I

Scarch

Max. A

Unnatched

Max. Popilide Masser

Scarch

Modificat by

Neuminus. Cerminus

Cerminus

Trypalo I serviamide: Hydrogeo (H)

Rece Acid (O H)

#### Result Symmary

k Uno	# alched	Sequence	MH+ Calculated (De)	MH Ecror (Da)	Proteio MW (Da)/pI	Species .	NCDIar:131002 Accession #	Protein Neme
1	0/9	(r) <u>allfydr</u> (r) (r) <u>allfydr</u> (r) (k) <u>ailfydr</u> (r)	815.5143 8(5.5143	Cioro. 2101.0-	846742/4.94 57068.0/6.28	Mus Musculus Homo Sapiens Homo Sapiens	123678 12804541	similar to hezz shock protein 86 90 kDz best-chock protein (AA 1-732) hypothetical protein LOC63929
-		the state of the last of the l		-	and the state of t			

### Detailed Results

						•			
Rink	# Unmaiched Ioni	Sequence	Mil" Calculated (Da)	(1/4)	Protein MW (Da)/pl	Species	NCBInt.121402 Accession 8	Index w	
1	0/9	(R)ALLEVER(B	3 815.5143	-0.10A3"	15541.0 / 5.28	MUS MUSCULUS	10881565	سينتين	similar to heat shock protein 86
-1	Q19	(R)ALLEYPR(F	815.5143	0.1043	84674,214.94	HOMO SATIENS	123678	1,63800	90 kDs hest-shock protein (AA I-732)
i	Fragment- Lon- Delta U9	ton (m/r) [185.26]  type   b <sub>1</sub>    D2   0.13    (K)AILFYPR(8    loa (m/r) [185.26]  type   b <sub>1</sub>	255.27 272. 9 <sub>2</sub> -NH <sub>3</sub> y <sub>2</sub> 0.12 0.17 0 815.514	0.11 0.11 1 -0.1043 34 298.3:	354.45 371,   Y <sub>3</sub> -NH <sub>3</sub>   Y <sub>3</sub>   0.24   0.25 57068.0 / 6.38	53 417.39 445.25 A <sub>4</sub>   b <sub>4</sub>	518.35 Y <sub>4</sub> 0.04 12R04541	<u>បោះ</u>	hypothetical protein LOC63929

### FIG. 13A

### MS-Fit Search Results

Sample 10 (comment): Alegic Bullet digest.
Database scarched: NCBInr.51403
Molecular weight search (1000 - 100000 Da) selects 1421445 entries.
Full pl range: (432416 entries.
Species search (HUMAN RODENT) selects 214836 entries.
Comblined molecular weight, pl and species scarches select 222557 entries.
MS-Fit search solects 5 entries (results displayed for top J matches).

Considered modifications: | Peptide N-terminal Gin to pyraGin | Oxidation of M (Pratein N-terminus Acceptated )

Min. # Peptides to Match	Peptide Mass Tolerance (+/-) 150,000 ppm	Peptide Marses are monoisotopic	Digest Used Trypsin	Mix. # Mixed Cleavagos	Cysteines Modified by acrylandde	Peptide N. teiminus Hydrogen (H)	Peptide C terminus Free Acid (O H)	Input # Peptide Masses
--------------------------------	---	--	---------------------------	------------------------------	---	--	--	------------------------------

#### Result Summary

Rabk	MOWSE Score	# (%) Masses Matched	Protein MW(Da)(p)	Species		Protein Name
£	1.81e+003	7/13 (53%)	94057.075.13	RATTUS NORVEGICUS	24075637	ischemia responsiva 94 kDa protoio
2 2			94133,1/5.15	MUS MUSCULUS MUS MUSCULUS	13277753 66kn301	heat shock protein 4 apg-2

#### Detailed Results

1. 7/13 matches (53%), 94057.0 Da. pt = 5:13. Acc. # 24025637. RATTUS NORVERICUS, isobemia responsive 94 kDa protein.

miz MHT Deltz start and Peptide Sequence Modifications submitted anarched ppm start and (N)VLTEVR(K)

798.5500 798.4514 123.4893 431 436 (K)VLTEVR(K)

949.6100 949.5219 92.7425 62 69 (K)NTVOGEKR(F)

1323.8500 1321.7116 104.7200 222 234 (K)VLATAFDITLGGR(K)

1402.7800 1402.6313 196,0213 619 629 (K)NAVEEYVYEMR(D)

1495.8400 1495.7029 91.6785 20 33 (R)AGGIETIANEYSDR(C)

1736.0700 1735.9271 82.3407 391 405 (R)EESITDVVFYPISLR(W)

1953.0400 1952.8336 105.6759 406 422 (R)WNSFAEEGSSDCEVFPK(N)

i unmarched masses: 915.6000 917.3600 1305;8400[1478,8800 1587,9500 1624,0500

The matched populates cover 10% (84/840 AA's) of the protein. Toverage Map for This Hit (MS-Digest Index 8); 787619

, 7/13 routches (53%), 9408 (, 1 Da, pt - 5;13, Acc. # 13277753, MUS MUSCULUS: heat shock protein 4.

### **FIG. 13B**

m/z MH Delta slart end Popude Sequence Modifications submitted in Alched ppm slart end Popude Sequence Modifications 198.5500 198.4514 123.4893 431 436 [K]VITEVR(K) 949.6130 949.5219 92.7415 62 69 (K)NTVOCFKR(P) 1305.8400 1305.7418 75.1849 670 680 (K)NVVVVVKLAFLK(S) 1321.8500 1321.7116 104.7200 222 234 (K)VLATAFDITLGGR(K) 1402.7800 1402.6313 106.0213 620 630 (K)NAVEFYVYEMR(D) 1495.8400 1495.7029 94.6785 20 13 (R)AGCIETIANEYSDR(C) 1736.0700 1735.9271 82.3407 391 405 (R)EFSITDVVPYPISLR(W)

6 unmarched masses: 915 6000 917.3600 1478.8800 1587.9500 1624.0500 1953.0400

The matched populdes cover 9% (78/84.1 AA's) of the protein. Coverage Map for This Hit (MS-Digest index #): 382745.

2. 7/13 matches (53%), 94133.1 Da. pt .. 5.15. Acc. # 6680301, MUS MUSCULUS, apg-2.

m/2 M/H<sup>+</sup> Della start end Peptide Sequence Modifications
798.5500 798.4514 123.4893 431 436 (K)YLTFYR(K)
949.6100 949.5210 92.7425 62 69 (K)NTVOCFKR(F)
(305.8400 1305.7418 75.1849 670 680 (K)OVYVDKLAELK(S)
1321.8500 1321.7116 104.7200 222 234 (K)VLATAFDTTLCCR(K)
1401.7800 1402.6313 106.0213 620 630 (K)NAVERYVYEMR(D)
1495.8400 1495.7029 91.6785 20 33 (R)ACCIETIANEYSDH(C)
1736.0780 1735.9271 82.3007 391 405 (R)EFSTFDVYTYYSLR(W)

5 unmatched masses: 915,6000 917,3600 1478,8800 1587,9500 1624,0500 1953,0400

The matched peptides cover 9% (78/841 AA's) of the protein. Tayerage Map for This Hit (MS-Digest index #): 1129227.

# **FIG. 14A**

#### MS-Fit Search Results

Sample ID (common): Magic Bullet digest
Database searched: NCB1 nr. \$1403
Molecular weight search (1000 - 100000 Ds) selects 1372760 entries.
Full pf range-1432416 entries.
Species search (170MAN RODENT) selects 224838 entries
Cambined inolecular weight, pf and species searches select 211465 entries.
MS-Pit search selects 335 entries (results displayed for top 5 matches).

Considered modifications: | Peptide Neterminal Can to hyroClu | Oxidation of M | Protein Neterminus Acctylated |

Min. # Peptides to Malch	Peptide Mass Tolerance (+V-) 150,000	Poptide Masses are monoisolopic.	Digesi Used Teypsin	Max: # Missed Cleavages 1	Cysteines Modified by scrylamide	Peptide N terminus Hydrogen (H)	Pepside C iominus Free Acid (O H)	Input # Peptide Musses 17
--------------------------------	--	---	---------------------------	------------------------------------	---	---------------------------------------	---	------------------------------------

#### Result Summary

Rank	MOWSE Score	#(%) Masses Malched	Protein MW (Du)(pt	Species	NCBInr.Si 403 Accession #	Protein Name
1	1.22=+005	11/17 (64%)	70149.7 / 5.20	MUS MÚSCULUS	29H40R03	unnamed protein product
2	1.226+005	11/17 (64%)	70163.8/5.24	MUS MUSCULUS	26326929	nuvauting bengin brognet.
. 2	2,66 <del>e1</del> 004	(0/17 (38%)	70201.8 / 5.28	Wing WingCOTAR	6679385	65-kDa macrophage protein

#### Detailed Results

11/17 malches (64%). 70149.7 Da. pt = 5.20. Acc. #29840803, MUS MUSCELLUS, unnamed protein product. start end Pentide Sequence WH+ Della za/z Modifications submitted matched ppm 700,4400 700,4146 36,2352 77 82 (K)VFHGLK(N) BILS200 BILA136 131.0872 585 591 (K)YAISMAR(K) 942.5800 942.5413 41.0831 442 449 (R)VNKPRYPK(L) 1069.7200 1069.6257 88.7285 264 272 (K)LSPERLLLR(W) 11267200 1126.6373 73.4015 433 441 (K)IKVYVDWNR(V) 1135.7100 1135.6111 87.0474 348 357 (R)OFVTATOVYR(G) 1287.7700 1287.6268 111.2008 402 412 (R)NWMNSLGVNPR(V) 1502.8900 1502.7525 91.5194 166 178 (K)MINLSVEDTIDER(T) 1585.9400 1585.8477 58.1710 597 610 (R)VYALPEDTVEVNPK(M) 1689.9700 1689.8560 67.4478 473 488 (K)FELVGIACODINECNR(T) 1758.0100 1757.8744 17.1528 310 326 (K)CHREGIFAVVIDMSCLR(E) unmarched masses: 927,5400 964,6000 1478,8800 1479,9200 1567,8600 1640,0900

he matched peptides cover 19% (120/627 AA's) of the protein.

### **FIG. 14B**

Coverage Map for This His (MS-Digest Index 4): 372729

2. 11/17 matches (64%). 70163.8 Da. pl = 5.24. Acc. # 26326929. MUS MUSCULUS, unnamed protein product.

on/z submitted	MH <sup>+</sup> mutched	bbin Delta	start	¢ពជ	Pentide Sequence	Modifications
700.4400	700.4146	36 2352	77	82	(K)VFHCLK(S)	-
811.5200	811.4136	131.0872	585	591	(K)YAISMAR(K)	
942.5800	942.5413	41.0831	442	449	(R)VNKPPYPK(L)	
1069.7200	1069.6257	88.1285	264	272	(K)LSPEELLIR(W)	
112G.7200	1126.6373	73.4015	433	441	(K)IKVPYDWNR(V)	
1135.7100	1135,6111	87.0474	348	357	(R)OFYTATOVVR(C)	
1287.7700	1287.6268	111.2008	402	.413	(R)NWMNSLGVNPR(V)	
1502.8900	1502,7525	91.5194	166	178	(K)MINLSVPDTIDER(T)	
1585.9400	1585.8477	58.1710	597	611	(R)YYALTEDLVEVNPK(M)	
1689.9700	1689.8560	67.4478	473	485	(k) FSLVCIACUDINEGNB(T)	i
(758:0100	1757.8744	77.1520	310	320	6 (K) <u>GDEEGIPAVVIDMSGLR</u> ()	<b>2)</b>

6 unmatched masses: 927,5400 964,6000 1478.8800 1479.9200 1567 8600 1640.0900

The matched peptides cover 19% (120/627 AA's) of the protein. Coverage Map for This Hit (MS-Digest index #): 1174311

3. 10/17 morches (58%). 70201.8 Da. pf = 5.28. Acc. Il 6679185. MUS MUSCULUS: 65-kDa macrophage protein.

```
start end Peptide Sequence
  zu/z
          MH<sup>+</sup>
                   Octa
                                                              Modifications
submitted matched
                   ppm
700.4400 700.4146 36.2352 77 82 (K)VFHCLK(T)
811,5200 811,4136 131,0872 585 591 (K)YAISMAR(K)
942.5800 942.5413 41.0831 442 449 (R)VNKPPYPK(L)
1069,7200 1069.6257 88.1285 264 271 (KOLSPRELLLR(W)
1126.7200 1126.6373 73:4015 433 441 (K)IKYPYDWNR(V)
1135,7100 1135,6111 87,0474 348 357 (R)OFVTATDVVR(G)
1287,7700 1287,6268 111,2008 402 412 (R)NWMNSLGVNPR(V)
1502.8900 1502.7525 91.5194 166 178 (K)MINLSVPDTIDER(T)
1585.9400 1585.8477 58.1710 597 G10 (R)YYALPEDLYLYNPK(M)
1758.0100 1757.8744 17.1528 310 326 (K)GDEEGIPAVVIDMSGLR(E)
```

1 unmarched masses: 927.5400 964.6000 1478.8800 1479.9200 1567.8600 1640.0900 1689.9700

the matched popules cover 16% (104/627 AA's) of the precion. Loverage Map for This Hit (MS-Digest index #): 746520

# FIG. 14C

#### MS-Tag Search Results

Sample 10 (continent): Api A-1 1040 AKPYLEDLR

Database exacted: NCBlan-51403

Alocalis weight search (1060 - 200,000 Da) selects 1421445 ethics.

Thil of page: 1437416 cipites.

Species ranch (HUMAN RODERN') selects 124818 enviet.

Species ranch (HUMAN RODERN') selects 124818 enviet.

Species ranch (HUMAN RODERN') selects 124818 enviet.

Number of squarence passing through parent mass filter: 1727

MS-Tex brongh selects 6 enviet.

Parent mass: 1287.7700 (14.0,2000 Da)

Fragman lans used in search: 175.00, 255.26, 172.20, 301:48, 369.17, 412.69, 542.65, 631.34, 655.97, 741.67, 840.69 (-4.0.70 Da)

Ion Types Considered: a b B y n h

Search Mal. Peptide Misses: Digiest Max. Missed Cynthes Peptide Peptide Mode Chemisched are Deed Chemisched Mode Chemisched In monatsolopite Tryptia 1 acrylanide Hydrogeo (H) Frée Arid (O R)

#### Result Sammary

. <u></u> gu	Lona Adicalcysy R	, Sequence .	MH <sup>2</sup> Calculated (Os)	MEC Ecror (Ds)	M.M. (Orlyb) Giotela	••	species	Meniman	Protein Name
1	•	(R)NWNINSLGVNPR(V)	1287.6268	0.1432	70288.8 / 5.29	HOI	MO SAPIENS	8117500	(Phiariu)) PVIIAH(T) (ilambrachte charolla beotefa j :
í	188	(R)NWMNSLGVNPR(Y)	1287.6158	0.1432	70289.7 / \$.10	но:	MO SAPIENS		tyrophocyte cytosolic protein I (L-plassic)
1		(R)NIVMANLGYMPROV		0.1433	70201:875.28	UM I	s musculus s musculus		65-KDs macrophage protein
1	{/}} 1/23	(R) WHITHSLOVERRY (R) YHITHNSLOVERRY	1287,6268	0.1432	70163.876.24	I MU	S MUSCULUS	26376919	unnamed protein product
ĩ	ιπ	(R)MWMMSLGVNPR(V	1287.6268	0,1432	70149.17.520	MU	is musculus	29840803	abortived brought blogging

#### Detailed Resulti-

Rusk	# Unmatched	Sequence	MIE* Calculated. (Da)	MH' Error (Da)	Protein MW (Daypi	Specter	HCBInt.SI403 Acception #	MS-Digest Index #	
	Ions	(R)NWMINSTGYNPR(Y		-	10288.8 / 5.29	HOMO SAPTENS	8217500	<u>696167</u>	bA (29H14, i (i) mphocyte cytosolic protein i (i, plastin))
		(K)KENNESS TELEST	•				4501965	225402	ijmphocyte cyrosolic protein l (L-plastin)
	•	MANUANCI CYNPRO	i . 1287.6268	0.1432	70201.8 75.28	MUS MUSCULUS	6679385	746520	63-kDa mactophago protein
1	vii.	MARKET CANALANT	1287.6268	0.1437	: 3333L-4 / B.60	MOS MOSCOLO	11947007	1145923	unnamed proisin product unnamed protein product
1	1/11	MINNEY CUNPTIO	A 1287.6268	0.1437	2. 70163.8 / 5.24	WDS WORCOTO	2 (0544585	1174311 171710	mustued bioleft brogner.
i	iru	(R)NWMNSLGVAPRO	A . 1287.626	0.143	1 70149.7 / 5.20	MUS MUSCULU		1	
	Fregment	-lou (m/z) [175,00] [155,28	272.30 301	48 369	.37 432.69 54	2,65 (633,34) (655.9)	742 0 7 0 4 0 . 0 V		
	lon	cope in lyz NII	0.13 (0.3)	10.11	mile the	liaz ibe	ليدة	<b>!</b> .	

# FIG. 15A

BLAST 2 SEQUENCES RESULTS VERSION BLASTP 2.2.4 Matrix BLOSUM62 El gap open 11 gap extension: x\_dropoff: 50 expect 10.00 wordsize: 3 Filter W Sequence I gi. 17865718 Heat shock prolein HSP 90-beta (HSP 84) (HSP 90) Length 724 (1 .. 724) Length 724 (1 .. 724) heat shock protein 84 - mouse Sequence 2 gi <u>72223</u> NOTE: The statistics (bitscore and expect value) is calculated based on the size of nr database Score = 1178 bits (3047) Expect = 0.0 Identities = 616/724 (858), Positives = 616/724 (85%) ndxxxxxxxxxtfafqaelaqlmsliintfysnkeiflreiisnasdaldkiryesit 60 query: 1 TFAFQARIAOLMSLIINTFYSNKEIFLRELISNASDALDKIRYESLT wberanncesearlayeovelyothattinlearnkeibluetienvedytokikaeepl eo sbjet: 1 DPSKIDSGKELKIDIIPNPQERTLILVDTGIGMTKADLINNLGTIAKSGTKAFMEALQAG 120 Query: 61 dpskldsgkelkidiipnpoertltlydtgigntkadlinnlgtiaksgtkapmealoag Sbjet: 61 DPSKLDSGKELKIDI1PNPDERTLTLVDTGLGMTKADLINNLGTIAKSGTKAFMEALQAG 120 Query: 121 ADISHIGQFGVGPYSAYLVAEKVVVITKHNDDEQYAWESSAGGSFTVRADHGEPIGRGTK 180 ad ismicofgvgfysaylvaekvvvitkompdeqyawessacceptvradhcepickcik Sbjct: 12.1 ADISMIGQFGVGPYSAYLVAEKVVVITKHNODEQYANESSAGGSFTVRADRGEPIGRGTK 180 **VILHUKEDQTEYLEERRVKEYVKKHSQFIGYPITLYLEK** Sbjct: 181 VILHLKEDOTEYLEERRYKEVYKKHSOFIGYPITLYLEKEREKEISDDEAEEEKGEKERE 240 XIDOEETNKIK I MILKU PODIL DEF Sbjet: 241 DKEDBEKOKIEDVGSDEEDDSGKDKKKKTKKIKEKYIDQEELNKTKPIWTRNPDDITOSE 300 Oncen: 301 AGELAKSPILLDMEDHITVAKHERABCOFELBYFIBEBYBEBYBYBKKKRNUTKTAAKKA 300 YGEFYKSLINDHEDHLAVKHFSVEGQLEFRALLFI PRRAPFOLFENKKKKHNIKLYVRRV Sbjec: 301 YGEFYKSLIMDWEDHLAVKHFSVEGQUEFRALLFIPRRAFFDLFENKKKOMIKLYVRRV 360 Query: 361 FIMDSCOELIPEYENFIRGVVDSEOLDENISREMLOGSKILKVIRKVIVKKCLELFSELA 420

### FIG. 15B

```
PINDSCDELIPEYLNFIRG SEDLPLNISREMLQQSKILKVIRKNIVKKCI.
Sbjcc: 361 FIMDSCDELIPEYLNFIRGVVDSEDLFLMISREMLQQSKILKVIRKNIVKKCLLLUFSELA 420
Query: 421 EDKENYKKPYERFSKNLKLGIHEDSTARRRLSELLRYHTSQSGDENTSLSEYVSRMKETQ 480
EDJC: 421 EDKENYKKFYERFSKNLKLGIHEDSTARRRLSELLRYHTSQSGDENTSLSEYVSRMKETQ 480
Query: 487 ksiyyitgeskequansafyervrkrofevvymtepideycvoqlkefdgkslvsvixxx 540
ksiyyitgeskequansafyervrkrofevvymtepideycvoqlkefdgkslvsvt
5bjc: 487 ksiyyitgeskequansafyervrkrofevvymtepideycvqqlkefdgkslvsvtked 540
I NLCKLMKEILDKKVEKVTISMRLVSSPCCIVTSTYGWTA
Sbjct: 541 LRLPEDEEEKKKMRESKAKFENLCKLMKEILDKKVEKVTISMRLVSSPCCIVTSTYGWTA 600
 Query: 601 nmerimkaqalednstmovėmakkelbinpdepivetlkokabadknokavkolvvlufe 660
              nmer imkaqalrdnətmoyrmakkhle inpdhp ivetlkokaradkydkavkolvvllfe
 Sbjet: 601 nmerimkagalrdnstmeyndakkhleinpdhpivetlrokaeadknokavkolvvllfe 660
 Tallssofsledpothsnriyrmiklglgi
                                                                     IPPLECOEDASRM
 Sbjct: 661 TALLSSGPSLEDPQTHSNRIYRMIKLGLGIDEDEVTAEEPSAAVPDEIPPLEGDEDASRM 720
 Query: 721 BBVD 724
               EBAD
 Sbjcc: 721 BEVD 724
                                            0.06 sys. secs
                                                                        0.21 total secs.
  CPU time:
                  0.15 user secs.
  sbdms.I
                0.135
     0.317
  Gapped
  Lambda
               0.0410
      0.267
  Matrix: BLOSUM62
  Gap Penalties: Existence: 11. Extension: 1
  Number of Hits to DB: 4890
  Number of Sequences: 0
   Number of extensions: 325
   Number of successful extensions; 1
   Number of sequences better than 10.0: 1
   Number of HSF's better than 10|0 without gapping: 1
   Number of HSP's successfully gapped in prelim test: 0
Number of HSP's that attempted gapping in prelim test: 0
Number of HSP's gapped (non-prelim): 1
length of query: 724
   length of database: 405,742,523
   effective HSP length: 134
   effective length of query: 590 effective length of database: $05.742,389 effective search space: 239388909510
    effective search space used: 239388009510
    T: 9
    A: 40
    X1: 16 ( 7.3 bits)
   X2: 129 (49.7 bits)
X3: 129 (49.7 bits)
    51: 41 (21.6 bits)
    s2: 78 (34.7 bits)
```

### **FIG. 16A**

# BLAST 2 SEQUENCES RESULTS VERSION BLASTP 2.2.4

Maurix BLOSUM62 E gap open: 11 gap extension: 1 x dropoff: 50 expect: 10.00 wordsize: 3 Filter F

Sequence I gi 72270 heat shock protein 86 - mouse Leugth 733 (1 .. 733) Sequence 2 gi 72223 heat shock protein 84 - mouse Length 724 (1 .. 724)





NOTE: The statistics (bitscore and expect value) is calculated based on the size of nr database

Score m 1102 bits (2851), Expect = 0.0 Identities = 561/733 (76%), Positives = 611/733 (82%), Gaps = 9/733 (1%)

- MPSETQTQDQPMESEEVETSAFQAELAQLMSLIINTFYSNKEIFTRELISHSSDALDKIR 60 Query: 1 EEEVETFAFQAEIRQLMSLIINTFYSNKEIFLRELISN+SDALDKIR
- mdeevhhg-----eeevetfafqaelaqlmsliintfysnkelflælisnasdaldkir 55 Sbjet: 1
- YESUTDPSKLDSGKELHINLIPSKODRILTIVDTGICHTKADLINNLGTIAKSGTKAPHE 120 Query: 61 Yeslydpsklosgkel 14+19+ Q+RTLT+VDTGIGMTKADLINNLGTIAKSGTKAFME
- YESLIDPSKLOSGKELKIDIIPNPQERTLTLVDTGIGMTKADLINNLGTIAKSGTKAFME 115 Sbact: 56
- Query: 121 ALQAGADISMIGQEGVGFYSAYLVAEKYTVITKENDDEQYAWESSAGGSFTVRTDTGSPM 180 ALQAGADISMIGQEGVGFYSAYLVAEKV VITKENDDEQYAWESSAGGSFTVR D GEP+
- Sbjct: 116 ALQAGADISMIGQFGVGFYSAYLVAERVVVITKHNDDEQYAWESSAGGSFTVRADHGEPI 175
- GRGTKVILHLKEDGTEYLBERR+KE+VKKHSQPIGYPITL++BKER
- sbjet: 176 grotkyluhlkedoteylberryktyvkyhsofigybitlylbkerbkbisdobabbekg 235
- LNKIKPIWIR
- -LNKTKPIWTR 191 Sbjet: 236 EKEEEDKEDEEKPKIEDVGSDEEDDSGKDKKKKTKKIKEKYIDQEE-
- Query: 301 NPDDITNEEXGEFYKSLTNDWEEHLAVKHFSVEGQLEFRALLFVPRRAPFDLFENRKKXN 360 NPDDIT EEYOEFYKSLINDWE+HLAVKHPSVEGQLEFRALLF+PRRAPFDDPEN+KKKN
- SDJCC: 292 NPDDITQEEYGEFYKSLTNOWEOHLAVKHFSVEGQDEFRALLFIFRRAFFDLFENKKON 351
- QUATY: 361 DIKLYVRRYFIMDNCEBLEBEYINFIRGVVDSEDLFLNISREMLQQSKILKVIRKNLVKK 420

### FIG. 16B

```
WIKLYVRRVFIMD+C+EL. :CHFIRGVVDSEDLPLWISREMLOOSKILKVI
                                                                  AMKK
Sbjct: 352 biklyvrvfimdscdeligeylnfirgvvdsedlplnisremlogskilkvir.nivkk 411
Query: 421 CCELFTELAEDKENYKKFYEQPSKNIKLGIHEDSQNRKKLSELLRYTTSASGDEMVSLKD 480
           CLELF+GLAEDKENYKKFYE PSKN+KLGIHEDS NR++LSELLRY+TS SGDEM SL +
Sbjct: 412 Clelpselædkenykkfybafskvlklgihedstvrrrlsellryhtsqsddemtelse 471
Quely: 481 yctrmkenqkhlyfitgetkdqvansafverlrkhcleviymiepideycvqqlkepegk 540
           y armke ok lyaltgs-kaovansarver-rk g evaym epideycvoolke7+Gk
Sbjct: 472 Yvsrmketoksiyyitgeskeqvansafvervrkrefevvymtefideycvqqlkefogk 531
+LVSVT
                                        NLCK+MK+IL+KKVEKV +SNRLV+SPCCI
Sbjct: 532 SLVSVTKEGLELPEDEE5KKKMEESKAKFENLCKLMKEILDKKVEKVTISNRLVSSPCCI 591
Query: 601 VTSTYGHTANMERIMKAQALRDNSTMGYMAAKKRLEINPDHSIIETLRQRAEADKNDKSV 660
           vtstygytanmerimkaqalirdnstmgym akkhleinpdh i+etlrqkaeadkodk+v
sbjct: 592 vtstygwtanmerimkaqalrdnstmgymhakkhleinpdhpivetlrqkaeadkndkav 651
Query: 661 KDLVILLYETALLSSGFSLEDPQTHAMRIYRMIKLGLGIDSDDPTVDDTSAAVTEEMPPL 720
           KDLV+LL+ETALLSSGFSLEDPQTH+NRIYRMIKLGLGIDED+ T ++ SAAV +E+PPL
Sbjot: 652 KDLVVLLFETALLSSGFSLEOPQTHSNRIYRMIKLGLGIOEDEVTABEPSAAVPDEIPPL 711
Query: 721 EGDDDTSRMEEVD 733
           EGD+O SRMEEVD
Sbjct: 712 EGDEDASRMEEVD 724
CPU time:
              0.17 user secs.
                                    0.01 sys. secs
                                                            0.18 total secs.
Lambda
           ĸ
   0.316
            0.134
                     0.372
Gapped
Lambda
           ĸ
   0.267 . 0.0410
                     0.140
 Matrix: BLOSUM62
 Gap Penalties: Existence: 11, Excension: 1
Number of Hits to DB: 5349
 Number of Sequences: 0
 Number of extensions: 384
 Number of successful extensions: 3
 Number of sequences better than 10.0: 1
 Number of HSP's better than 10.0 without gapping: 1
 Number of HSP's successfully gapped in prelim test. 0
Number of HSP's that attempted gapping in prelim test: 0 Number of HSP's gapped (non-prelim): 1
 length of query: 733
 length of database: 405,742,523
 effective HSP length: 134
 effective length of query: 599
 effective length of database: 405.742,389
 effective search space: 243039691011
 effective search space used: 243039691011
 T: 9
 A: 40
 X1: 16 ( 7.3 bits)
 X2: 129 (49.7 bits)
 X3: 129 (49.7 bital
 S1: 41 (21.6 bits)
 S2: 78 (34.7 bics)
```